bio-protocol Preprint

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ChIP-Sequencing

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An abbreviated version of this protocol was published in eLIFE in Oct 2019

Herpes simplex viral nucleoprotein creates a competitive transcriptional environment facilitating robust viral transcription and host shut off

DOI: 10.7554/eLife.51109

Detailed protocol

Adapted from Myers Lab ChIP-seq Protocol v011014
Johnson *et al*., 2007, Science 316: 1497- 1502

Reagents:

- cOmplete protease inhibitor cocktail pills (Sigma # 11697498001)
- Large TC Dish 245x245x25 (ThermoFisher #166508)
- DMEM, powder, high glucose, pyruvate (ThermoFisher #12800017)
- Dynabeads M-280 Sheep anti-Mouse IgG (Invitrogen #11201D) or Dynabeads M-280 Sheep anti-Rabbit IgG (Invitrogen #11203D)
- DynaMag-2 (Life Technologies #12321D)
- BSA (Fisher #BP1605-100G)
- QIAquick PCR Purification Kit (Qiagen #28104)
- DNA LoBind Tube (Eppendorf #022431021)
- Qubit™ dsDNA HS Assay Kit (ThermoFisher #Q32851)

Buffers:
**Autoclave solutions to sterilize*
Tricine-buffered saline (TBS) (store at RT)
137 mM NaCl
5 mM KCl
0.5 mM MgCl
0.7 mM CaCl
25 mM Tricine
Adjust pH to 7.35 with 10 N NaOH

1x Phosphate-buffered saline (PBS) (store at RT)
137mM NaCl
2.7 mM KCl
10 mM Na2HPO4
1.8 mM KH2PO4
Adjust pH to 7.2 with 10 N NaOH

1x TE (store at RT)
10 mM Tris
1 mM EDTA

**Sterile filter solutions with 0.2um filter*
Quenching Solution (store at RT)
2.5 M Glycine

Farnham Lysis Buffer (FLB) (store at 4 degrees)
5mM PIPES (Piperazine-1,4-bis(2-ethanesulfonic acid)) pH 8.0
85mM KCl
0.5% IGEPAL (N3500 Nonidet-P40 substitute CAS #9036-19-5)

RIPA Buffer (store at 4 degrees)
1xPBS
1% IGEPAL (N3500 Nonidet-P40 substitute CAS #9036-19-5)
0.5% Sodium Deoxycholate
0.1% SDS
+Protease inhibitors the day of use

LiCl IP Wash Buffer (store at 4 degrees)
100mM Tris-HCl pH 7.5
500mM LiCl
1% NP-40
1% Sodium Deoxycholate

BSA/PBS (store at 4 degrees)
5mg/mL BSA
1x PBS

Infection:
**In 245x245x25 tissue culture plates there are approximately 7 x 107 MRC5 cells at confluence. Make sure to plate and growcells at 37°C until they are completely confluent*
To infect at an MOI of 10 infect with 108 PFU/mL, 7mL/plate

- Dilute appropriate virus in cold TBS
- Aspirate media from plates
- Add on 7 mL of viral inoculum
- Adsorb for 1 hour at room temperature (RT), rock gently every 10 minutes
- Prep media and TBS, and place in 37°C water bath to prewarm
  - Prep 50 mL 2% FBS 1X DMEM media per plate
  - Prep 50 mL TBS per plate
- Aspirate viral inoculum
- Wash with 50 mL prewarmed TBS
- Add 50 mL prewarmed 2% FBS 1X DMEM media
- Incubate at 37°C until harvest

Cross-linking and harvesting:
**All future steps should be performed on ice, and all solutions should be kept chilled.*

- Prep solutions day of use:
  - 25% formaldehyde
    - Dilute stock 36.5% formaldehyde in water
    - Need 5mL per plate
  - FLB + 1x Protease Inhibitor (PI) pills
    - 1 PI pill dissolved per 50 mL solution
    - Need 50mL solution per plate
- Add 5 mL 25% formaldehyde to plate, swirl to mix
- Incubate RT 10 min
- Add 5mL quenching solution (2.5 M glycine), swirl to mix
- Aspirate medium from plates
- Add 50 mL cold TBS
- Aspirate wash completely
- Add 50 mL cold FLB + PI to each plate
- Scrape cells using large scraper into 50 mL conical
- Spin at 2500 rpm 10 min at 4°C
- Aspirate supernatant (SN)

**If necessary can flash freeze pellet in liquid nitrogen and store at -80°C until on Day 2 of the protocol. Alternatively can aspirate SN and immediately add 1.1mL RIPA+PIs, starting on step 4 of the Day 2 protocol.*

Day 1
Equilibrate beads:

- Combine 50 µL Dynabeads M-280 Sheep anti-Mouse or anti-Rabbit magnetic beads with 1mL 5mg/mL BSA in 1x PBS
- Place on Dynamag-2 magnet for 2 minutes, aspirate SN
- Wash with 1mL 5 mg/mL BSA in PBS (x3)
- Resuspend in 1 mL BSA/PBS

Bind primary antibody to beads:

- Add 5-25 µg primary antibody to magnetic beads
- Rotate overnight at 4°C

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Day 2
Sonication:

- Prep solutions day of use:
  - FLB + 1x Protease Inhibitor (PI) pills
    - 1 PI pill dissolved per 50 mL solution
    - Need 50mL solution per plate
  - 1x RIPA 1x PI's
    - 1 PI pill dissolved per 50 mL solution
    - Need 1.1mL solution per plate
- Thaw nuclear pellets on ice.
- Resuspend pellet gently in 4mL FLB + PI (invert tube to dislodge pellet, want to keep the pellet intact!)
- Spin 2500rpm 10min 4°C, aspirate SN
- Resuspend pellet gently in 1.1mL RIPA + PI. (Again do not breakup pellet, just dislodge from tube).
- Transfer pellet into 1.5mL microfuge tube (I use a 2mL pipette to suck up the intact pellet).
- Pipette up and down or vortex to break up pellet.
- Sonicate 6 x 30sec, power 40%, do in ice bath (3 min total pulse time)
  - Ensure solution is homogenous without any fragments floating
- Spin 14000rpm 4°C 15min
- Aliquot 50uL SN into screwtop tube and place at -80°C to use as "input"
- Split remaining volume of nuclear lysate equally for the number of IP's per sample
  - For two antibodies: aliquot 500uL of each sample

Immunoprecipitation:

- Wash beads from Day 1 with 1mL 5 mg/mL BSA/PBS (x3)
- Resuspend beads in final volume of 100uL 5 mg/mL BSA/PBS
- Add 100 µL beads to each nuclear lysate sample
- Rotate overnight at 4°C

Day 3
Wash beads

- Prep solutions day of use:
  - 2x IP Elution Buffer
    - 2% SDS
    - 0.2M $NaHCO_3$
  - 1x IP Elution Buffer
    - 1% SDS
    - 0.1M $NaHCO_3$
- Place microfuge tubes on magnet for 2 min and aspirate SN
- Wash with 1mL LiCl wash buffer 3 min rotating 4°C (x7)
- Wash with 1mL 1x TE 1 min, place on magnet

Elute beads

- Aspirate SN, resuspend in 200uL 1x IP elution buffer. Vortex well
- Incubate 65°C 900rpm 2hrs
- Spin 3min 12000 rpm RT, magnet. Transfer SN to screwtop tube.
- Add 50uL 1x TE + 100uL 2x IP elution buffer to the 50uL aliquot of input.
- Incubate Input and IP Chromatin at 65°C 900 rpm O/N

Day 4
Extract and purify DNA

- Add equal volume (200uL) phenol:chloroform:isoamyl alcohol (25:24:1) to samples
- Spin 12000 rpm 15 min RT
- Transfer top, aqueous phase to new tube
- Back extract: add 50uL water to lower phase in original tube
- Spin 12krpm 15 min RT
- Combine top, aqueous phase with previously extracted phase
- Add equal volume (200uL) chloroform:isoamyl alcohol (24:1) to samples
- Extract with 200uL Chloroform:Isoamyl alcohol (24:1)
- Spin 12krpm 5 min RT
- Transfer top, aqueous phase to new tube
- Purify with Qiagen PCR cleanup column

- In first step make sure to add 10uL NaOAc pH 5.0
- Elute with 60uL buffer EB into DNA LoBind Tube

- Measure DNA using Qubit 2.0 and the Qubit™ dsDNA HS Assay Kit

Library Synthesis
Reagents:

- NEB Ultra II DNA Library Prep Kit w/ Beads (NEB #E7103S)
- NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 1) (NEB #E7335S)
- Magnetic Stand-96 (Ambion RNA by Life Technologies #1111049)
- Multiplate PCR Plates 96-well, clear (Bio-Rad #MLP9601)
- Microseal 'B' seal Seals (Bio-Rad #MSB1001)
- Agilent DNA 7500 Kit Agilent # 5067-1506

Follow NEBNext® Ultra™ II DNA Library Prep Kit for Illumina instructions (manualE7645)

- Generally use 20 ng Input DNA, and up to 20 ng of IP DNA
- Perform size selection (Step 3A), selecting for approximate final library size of 270 bp
- Assess concentration and purity using Agilent DNA 7500 Kit (Agilent #5067-1506)

Related files

ChIP-Seq.pdf

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. DeLuca, N. (2019). ChIP-Sequencing. Bio-protocol Preprint. bio-protocol.org/prep128.
2. Dremel, S. E. and DeLuca, N. A.(2019). Herpes simplex viral nucleoprotein creates a competitive transcriptional environment facilitating robust viral transcription and host shut off. eLIFE. DOI: 10.7554/eLife.51109